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MECHANISMS OF COORDINATION BETWEEN MOULTING AND REPRODUCTION IN TERRESTRIAL ISOPOD CRUSTACEA

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It has long been believed that both moulting and egg development in decapod Crustacea are under inhibitory hormonal control by neurosecretions released from the sinus gland (Gabe, 1966; Adiyodi and Adiyodi, 1970; Sochasky, 1973). The observation of apparently synchronous moulting and reproduction in adult crabs (Panouse, 1947) led initially to the view that the processes were "synergistic" and that a single hormone might control both. This view subsequently gave way to the concept of "antagonism" between somatic and reproductive growth, according to which one process occurs only at the expense of the other (Passano, 1960; Charniaux-Cotton and Kleinholz, 1964; Bliss, 1966; Adiyodi and Adiyodi, 1970; Sochasky, 1973). Both concepts developed from consideration of the temporal relations between moulting and reproduction among decapods; their applicability to other groups of the Crustacea is not clear.

The present paper examines the mechanisms which coordinate and regulate reproduction and moulting in terrestrial isopods. In the laboratory, as in the field (Heeley, 1941), these animals moult frequently and regularly and can produce several broods of young per year. Moulting and breeding in decapods maintained in the laboratory are generally less frequent and less regular. Information on control of moulting and reproduction in isopods is based primarily on descriptions of the effects of ablation and transplantation of the protocerebrum (Reidenbach, 1965; Bessé, 1968; Bessé *et al.*, 1969; Mocquard *et al.*, 1971; Bessé and Donady, 1972; Charmantier and Trilles, 1975). The conflicting results reported may be related to lack of information concerning the relationships between these processes in normal animals and the consequent inability to perform surgical manipulations at appropriate times. The present paper examines these relationships in normal isopods and shows that moulting and reproduction are controlled by separate environmental cues and probably separate hormones. However, the relationships are more subtle than terms such as "synergism" and "antagonism" imply; rather, moulting and reproduction are coordinated by specific sensory cues which maintain precise, if complex, temporal relations between them.

Materials and Methods

All of the observations and experiments described have employed *Oniscus asellus* (L.). Many of them have been repeated using *Porcellio spinicornis* (Say), *Cyclisticus convexus* (De Geer) and *Trachelliscus rathkei* (Brandt). The conclusions reported are applicable to all these species; however, quantitative differences were found between species, mainly in the total length of the intermoult cycle. The quantitative data reported are those for *Oniscus asellus*. The experiments reported for *Oniscus* have been repeated, and have used both animals born and raised in laboratory cultures and animals collected from the field in early spring before either moulting or reproduction has commenced. All animals used in

experiments had a head capsule width of 2.0–2.5 mm. Animals this size in the field are 1-year-olds which have not reproduced (McQueen, 1976). Head capsule width appears to be the most reliable convenient parameter of age (Standen, 1970). Body weight was not used, as terrestrial isopods undergo substantial changes in water content (Lindqvist, 1972; Mayes and Holdich, 1975).

Laboratory cultures were maintained at 21 ± 1°C in a 16L : 8D daylength cycle. An individual culture consisted of 6–20 animals in a plastic Petri dish about 9 by 2.5 cm equipped with a humidity wick, crushed limestone from the vicinity of the field collections, and ground Purina rabbit chow, which Merriam (1971) found to provide an optimum diet. Females incubating broods of young were isolated in single culture dishes until the young were liberated. The adult was then removed and the young raised together in the dish. Thus the age of laboratory-born animals was known precisely.

The procedure for determining the various stages of the moult cycle will be described in detail elsewhere (Steel, in prep.). In brief, the sternites of pereion segments 1–4 undergo systematic changes in appearance due to the accumulation of fat cells and calcium deposits, which develop through a characteristic sequence of changes in shape and size at specific times during the cycle. Fourteen distinct configurations are recognizable on brief visual inspection of the animal's ventral surface. Stages 1 through 9 represent premoult. The same 14 stages occur with the same relative durations in all species examined, despite species variation in total cycle length. These stages are correlated with the changes associated with secretion and resorption of the exoskeleton (unpublished observations) and are readily related to the alphabetical subdivisions of the moult cycle used in decapods (Drach, 1939). Being the more familiar, the latter terminology is used in this text. The term premoult is used to refer to the period between moult initiation and ecdysis (Stage D in the terminology of Drach). Postmoult is the period of post-ecdysial secretion and calcification of the exoskeleton (Stages A, B, and C_1–C_3) and intermoult is the period between the end of postmoult and initiation of the next premoult (Stage C_4). Durations given in the text are means ± S.D.

Results

Differential effects of temperature and daylength

Observations on field populations have shown that moulting occurs throughout the summer, whereas females incubating broods of eggs are found only during a part of this season (see McQueen, 1976, for details). These observations imply distinct seasons for each process. This in turn suggests separate environmental regulation of moulting and reproduction. This possibility was examined in the laboratory using both field-collected and laboratory-raised animals of the same size. Females from both sources moulted regularly at intervals of 28 days at 21 ± 1°C. Moulting in males was slightly less frequent and less regular (see Table II). Moulting frequency exhibited a Q_{10} of two between 12° and 22°C and was arrested completely at 5°C. Animals maintained in intermoult at 4°C could be induced to moult by transferrance to 21°C. In such animals, the first sign of of early premoult occurs 14 ± 1.9 days after transfer to 21°C. Animals brought in from the field in either spring or autumn from outside temperatures of 0–5°C responded to this increase in temperature in the same way. These observations suggest that premoult is initiated following a threshold number of warm days. Parallel cultures maintained under "short" (8L : 16D) or "long" (16L : 8D) daylength regimes continue

TABLE I

Effect of daylength on induction of breeding in female Oniscus. *In long days the 17% which did not breed at the first moult all did so at the second. N = 40. See text for details.*

Daylength regime	Long days 16L:8D		Short days 8L:16D	
Type of moult	Breeding	Non-breeding	Breeding	Non-breeding
First moult	83%	17%	9%	91%
Second moult	73%	27%	27%	73%

to moult throughout the year with the same frequency (except under conditions which stimulate vitellogenesis, as described below). Thus, moulting is strongly temperature dependent but is apparently independent of daylength.

Adult females are capable of two different types of moults. One is similar to that of males and no change in form occurs. The other occurs only during the breeding season in the field and is morphogenetic in the sense that the oostegites of pereion segments two to five enlarge enormously to form a brood pouch in which eggs are incubated ("maternal" moult of Heeley, 1941). The influence of daylength on the morphogenetic character of the moult was examined in the following experiment. Twenty female *Oniscus* which had previously been held in intermoult at 4°C were induced to commence moulting by transferrance to 21°C. Half were placed in 8L : 16D and half in 16L : 8D. A further 20 intermoult females collected from the field in early spring were also set up under these two conditions. The proportions forming brood pouches at each of the first two moults are shown in Table I. No differences between laboratory-bred and field-collected animals were detected. Accordingly, Table I presents the combined data for all 40 animals in each regime. In long days, almost all animals formed brood pouches at the first moult, and of those that did not, all did so at the second moult. Accordingly, the 27% of 40 animals which underwent a non-breeding second moult in long days had all undergone breeding at the first moult. The majority of animals in long days underwent two breeding moults in succession. Conversely, breeding was rare in the short-day animals, even after two moults. Thus, while the occurrence of moulting appears to be independent of daylength, the morphogenetic character of the moult is strongly influenced by daylength.

The interaction of temperature with daylength in *Oniscus* has been discussed in detail by McQueen and Steel (1980). It is sufficient here to note that temperature does not directly affect whether or not reproduction will occur; the role of temperature in regulating reproduction appears to be confined to determining the rapidity with which animals respond to those daylengths which promote reproduction due to the influence of temperature on the length of time between ecdyses. Thus, temperature affects moulting but has no direct effect on reproduction. Daylength and temperature, therefore, exert differential effects on moulting and reproduction.

Breeding cultures can therefore be maintained in the laboratory at 21°C in "long" days. Although moulting continues throughout the year under these conditions, females do not breed throughout the year. These laboratory cultures cease breeding in September in synchrony with field populations, indicating that the cessation of breeding is brought about by different cues from those which induce

it. For the following 3 months females remain refractory to the stimulation of reproduction by long daylengths, but become responsive to them again in December, when field populations are completely dormant. Thus, there appears to be a seasonal periodicity in responsiveness to conditions promoting breeding. The absence of such periodicity influencing moulting further supports the view that moulting and reproduction are controlled by separate mechanisms which are differentially influenced by environmental factors.

Stages of oogenesis

Mature eggs are deposited into the brood pouch a few hours after ecdysis (Nemec, 1896). The number of eggs per brood pouch averaged 38 ± 6. In order to examine the interrelations between the development of these eggs and the moulting cycle, it is necessary to recognize the different stages of egg development. The paired ovaries are tubular structures extending throughout the pereion. In non-breeding animals, each ovary contains 19 ± 8 small white oocytes 100–200 μm (mean 131 μm) in diameter. These correspond in size and appearance to the "previtellogenic" oocytes of the amphipod *Orchestia gammarella* (Charniaux-Cotton, 1973). Thus, each animal contains sufficient previtellogenic oocytes for the production of exactly one brood of young without cell division. These oocytes do not change in size, appearance, or number during moult cycles which do not lead to formation of a brood pouch. It therefore seems that oocyte development is arrested in the "previtellogenic" stage until reproduction is stimulated.

When reproduction is stimulated, all the previtellogenic oocytes grow synchronously from 200 to 600 μm. While this is occurring they become progressively more yellow. Globules accumulate in the cytoplasm and increase in size and number with growth of the oocytes. These changes in oocyte size and appearance are commonly regarded as due to the accumulation of yolk. Hence, this phase of oocyte development represents vitellogenesis. That vitellogenesis does indeed occur in *Oniscus* oocytes at this stage is confirmed by the finding that developing oocytes first acquire immunoreactivity to antiserum raised against purified *Oniscus* yolk proteins at a size of 150–200 μm (C.G.H. Steel and R. A. Baron, unpublished observations). Mature eggs newly deposited into the brood pouch are similar in size and appearance to the largest seen in the ovaries.

During the incubation of a batch of mature eggs in the brood pouch, oogonia proliferate and previtellogenic growth of oocytes occurs, thereby restoring the complement of previtellogenic oocytes by the time the brood is liberated. Brood

TABLE II

Modification of chronology of moult cycle by egg development and incubation. The postmoult period lasts 2–3 days. Hence intermoult is considered here to begin 2 days after anterior ecdysis and to end with initiation of premoult. Thus, brood incubation time equals length of intermoult plus postmoult.

	Length of premoult	Length of next intermoult	No. of animals
Males	16.3 ± 2.0 days	10.0 ± 5.1 days	25
Females, non-breeding	16.2 ± 1.8	7.1 ± 1.4	29
Females, first brood	33.0 ± 3.3	32.0 ± 2.6	25
Females, second and subsequent broods	17.7 ± 3.1	33.8 ± 3.7	31

incubation lasts until the young hatch within the brood pouch and crawl out of it. At 21°C the average duration of brood incubation is about 34 days (see Table II), longer than a complete intermoult cycle in non-breeding animals. The mechanisms by which growth of the oocytes and brood incubation are coordinated with moulting activities are examined below.

Effects of reproduction on moulting

As noted above, brood incubation takes longer than a complete intermoult cycle of non-breeding animals. This implies that the chronology of the intermoult cycle is modified during brood incubation.

Since deposition of mature eggs into the brood pouch occurs shortly after ecdysis, incubation commences during the postmoult period. Females incubating eggs were inspected every 3 days for signs of premoult. No premoult was initiated until the end of brood incubation (see also below). This confirms that brood incubation occurs during the intermoult stage (Stage C_4 of Drach, 1939). However, the postmoult and intermoult stages of non-breeding females together last only about 9 days (Table II), whereas, incubation lasts about 34 days at the same temperature. Therefore, initiation of premoult must be delayed about 25 days in females incubating a brood. Thus, brood incubation influences the moult cycle by causing an extension of intermoult from 7 to 32 days (Table II).

Previtellogenic growth of the oocytes occurs during brood incubation, *i.e.*, during postmoult and intermoult. These oocytes undergo fertilization and incubation following a subsequent ecdysis and formation of another brood pouch. Thus, at least one moult occurs between previtellogenic growth and fertilization. This arrangement contrasts with that in higher decapods in which both egg maturation and incubation occur within a single intermoult (Adiyodi and Adiyodi, 1970).

Reproduction also modifies the chronology of premoult. This period lasts 16 days in non-breeding *Oniscus* females at 21°C (Table II). However, in animals kept in intermoult at 4°C, and then transferred to 21°C and long days to stimulate a maternal moult, the premoult period lasts 33 days (Table II). All the constituent sub-stages were found to be of their normal length except for Stage 3 (late D_0 in the nomenclature of Drach, 1939) which was extended from 3 to 20 days in duration. Thus, there is a lengthy pause in the early premoult of animals stimulated to undergo a first maternal moult. Dissection of the ovaries of animals which had been in this protracted late D_0 for various known lengths of time revealed that the changes in size and appearance of the oocytes which accompany vitellogenesis commence during this period. Moreover, the oocytes first develop immunoreactivity to anti-vitellogenin serum at this stage (C. G. H. Steel and R. A. Baron, unpublished observations).

Thus, vitellogenesis commences in early premoult, at which time the moulting process undergoes suspension for 16 days, at the end of which premoult changes resume with the same chronology as in non-breeding females. However, vitellogenesis is not completed during the period of premoult suspension. Dissection of ovaries from females at various stages of premoult showed that oocyte growth is not completed until shortly before ecdysis (Table III) and most of the increase in size and accumulation of yellow globules in the oocytes occurs after the premoult has resumed. Suspension of premoult is therefore not necessary throughout vitellogenesis.

TABLE III

Chronology of vitellogenesis in first and second maternal moult cycles. The oocyte size ranges given indicate the average size increases observed between the beginning and end of the moult stage indicated. Oocyte sizes were measured for 30 oocytes (15 from each ovary) from each animal. "n" gives the number of animals dissected at each stage.

Stage of moult cycle	First maternal moult				Second maternal moult			
	n	Oocyte size range	Oocyte appearance	Stage duration	n	Oocyte size range	Oocyte appearance	Stage duration
Intermoult								
Early	11	100–200 μ	Clear, white	7 days	17	100–200 μ	Clear, white	24 days
Late					19	200–300 μ	Opaque, yellowish	8 days
Premoult								
Early (D_0)	12	200–250 μ	Opaque, yellowish	24 days	12	330 μ	Opaque, yellowish	8 days
Late (D_1–D_3)	16	250–600 μ	Opaque, yellow	9 days	8	330–600 μ	Opaque, yellow	9 days

A further illustration of interactions between reproduction and moulting is seen when females incubating broods of eggs are maintained in environments promoting reproduction, such that two or more maternal moults occur in succession, a new brood pouch being formed under the old one during the premoult following liberation of the brood. Under these conditions, the pause in late D_0 seen in the first maternal moults is absent (Table III). The chronology of all stages of premoult is the same as in non-breeding females. Dissection of the ovaries of these animals revealed that at the beginning of premoult, the oocytes had already developed to an average size of 330 μm (Table III) and had accumulated significant numbers of yellow globules. This indicates that the timing of vitellogenesis in the first maternal moult is different from that seen in subsequent maternal moults. During incubation of the first brood, the oocytes do not cease growth at the end of the previtellogenic stage, but continue into vitellogenesis, such that by the time the brood was liberated the oocytes were about halfway through the changes in size and appearance associated with vitellogenesis (Table III). Thus, the premoult following brood liberation commences with the oocytes already undergoing vitellogenesis. However, vitellogenesis is completed in late premoult, as in first maternal moults. In other words, the later part of vitellogenesis invariably occurs in late premoult, whereas the first part can occur either in early premoult (first maternal moult) or during intermoult (second and subsequent maternal moults). Whichever stage vitellogenesis commences in undergoes a considerable extension relative to its duration in non-breeding animals; the extension to intermoult during incubation is 25 days and the extension to early premoult seen in first maternal moults is 16 days. Thus, the total time occupied by vitellogenesis is similar in both first and subsequent maternal moult cycles, but the position of the first part of vitellogensis in the moult cycle is flexible.

Stimulus to premoult initiation

The finding that intermolt is protracted during incubation suggested the possibility that the timing of the initiation of the next premoult might be regulated by some event associated with incubation. Subsequent to fertilization of the eggs, the role of the mother in brood incubation seems to be confined to production of a watery fluid which surrounds the eggs in the brood pouch, for fertilized eggs can be grown *in vitro* in saline (Sutton, 1972). The eventual liberation of the young from the pouch "seems to be simply a matter of the young crawling out when they are ready" (Sutton, 1972), over a period of up to 2 days (Heeley, 1941). The oostegites are held in a distended position by the brood, but collapse to the sternites when the young are liberated. The first visible signs of premoult are seen 3.1 ± 0.8 days after the complete collapse of the brood pouch.

Premature deflation of the brood pouch was produced by rinsing away the developing eggs or embryos in a fine stream of tap water. This resulted in moult initiation, premoult becoming visible on the third day following collapse of the brood pouch as in normal animals. If one or two eggs remained stuck in the pouch, the appearance of premoult was greatly delayed, usually by about 10 days. Thus, the presence of eggs in the brood pouch is necessary to maintain the intermoult condition. In the converse experiment, the brood pouch of females which had just liberated young was stuffed with cellulose sponge moistened with the brood pouch saline of Sutton (1972). No initiation of premoult was seen in these animals. When the sponge was removed after about 10 days, premoult was detectable after 3 days as if a normal brood had been liberated.

The initiation of premoult is therefore determined by the time of collapse of the oostegites. Presumably, proprioceptive or tactile information from these appendages provides a nervous pathway for regulating release of the moult-controlling hormones. These observations illustrate quite strikingly that events associated with reproduction provide cues which regulate both length and timing of the moult cycle.

However, in males and in non-breeding females, premoult initiation must be accomplished by a mechanism different from the above. The role of a threshold number of warm days has been mentioned earlier. However, the length of intermoult is remarkably constant, especially in females, even after months of constant temperature and daylength. Even nutritional state seems not to be fundamental, for starved animals continue to moult frequently even though they become smaller at each moult. It is clear that moulting does not occur solely to permit growth. However, the mechanism of regular premoult initiation in a constant environment remains unknown.

Discussion

Environmental regulatory factors. Both temperature and daylength influence moulting and reproduction, but have differential effects on the two processes. Moulting is strongly temperature dependent but is unaffected by daylength (McQueen and Steel, 1980). Since moulting ceases at 5°–10°C, moulting in field populations should occur when temperatures reliably exceed 5°–10°C. Local meteorological records show such field temperatures between March and October. Conversely, reproduction is induced by long days (16L:8D) and suppressed by short (8L:16D) in regularly moulting animals. This effect appears to be a

conventional photoperiodic response with a critical daylength of about 11.5 hr (McQueen and Steel, 1980). At 44°N, daylength would promote reproduction in the field between April and September. These expectations are confirmed by field observations on natural populations of *Porcellio* in the same vicinity (McQueen, 1976) and of *Oniscus* in England (Heeley, 1941). Thus, daylength confines reproduction to the central portion of the season when temperature permits moulting. It is concluded that moulting and reproduction are confined to their respective seasons by differential responsiveness of the two processes to the environmental cues of temperature and daylength. Such differential responsiveness further suggests the existence of separate physiological regulatory mechanisms for each process, as discussed below.

Superimposed on these effects of temperature and daylength is a seasonal periodicity in the responsiveness of females to environments which promote breeding. Females become refractory to long-day stimulation in the laboratory at about the time when breeding ceases in the field, and remain so for the following 3 months. Similar events occur in cultures of *Porcellio* in France (Bessé and Maissiat, 1971). This refractoriness suggests the intervention of a phenomenon analogous to reproductive diapause. The absence of seasonal periodicity in moulting in the laboratory supports the above inference that moulting and reproduction are separately controlled.

Stimulus to moult initiation. Brood incubation occurs during an intermoult which is prolonged to about four times its duration in non-breeding moult cycles; initiation of the next premoult is delayed until the young are liberated from the brood pouch. The collapse of the brood pouch when the brood is liberated provides a trigger which initiates premoult. Three days elapse before the appearance of the first morphological signs of premoult. Since morphological changes result from prior hormonal changes, it must be in this period between the stimulus and the appearance of morphological changes that the endocrine events associated with premoult are initiated.

All other descriptions of stages of moult cycles in Crustacea rely on the appearance of some tissue change to define the beginning of premoult. Such descriptions of premoult in terms of effects rather than causes inevitably overlook the initial stage(s) of premoult in which key physiological changes occur. Thus, animals would be inappropriately classified as in intermoult. This may account for the high individual variation in indices of metabolic activity noted previously in ostensibly intermoult animals (*e.g.*, Andrieux, 1979). The proportion of the total premoult period which passes without morphological change in *Oniscus* is 3 out of 16 days at 21°C, or 19%. However, most other crustacean moult staging schemes recognize premoult by the occurrence of apolysis in the integument (Drach and Tchernigovtzeff, 1967). In *Oniscus*, apolysis occurs in different regions of the integument between 5 and 8 days after the moult-initiating stimulus (unpublished observations). Thus, if premoult were recognized by apolysis in *Oniscus*, as it is in *Sphaeroma* (Tchernigovtzeff and Ragage-Willigens, 1968), the proportion of the premoult period which would pass unnoticed would increase to as much as 50%.

It is inferred that the stimulus which initiates premoult and the primary hormonal changes occurs before tissue changes become evident, and certainly well before apolysis. Thus, premoult begins well before it is recognizable by conventional moult-stage schemes. This conclusion lends substance to that of Passano (1960), who presumed that early D_0 would not be morphologically detectable, but differs

strikingly from that of Reaka (1975) and Vranckx and Durliat (1978), who propose that initiation of premoult may not occur until *after* apolysis.

Coordination of moulting and reproduction. The mechanism discussed above, whereby intermoult is extended during incubation, is but one of several mechanisms which coordinate the moult cycle with reproductive events.

The point in the moult cycle at which vitellogenesis commences was found to be flexible. During a first maternal moult it commences in late D_0 and is accompanied by a temporary suspension of this stage of premoult for 16 days, resulting in a premoult period of double the normal length. In animals in which a second maternal moult follows the first, this prolongation of premoult is not seen and vitellogenesis commences during the preceding intermoult. Thus, different temporal relations are found between vitellogenesis and the moult cycle under different conditions, which again suggests that separate mechanisms control these processes. However, regardless of the moult stage at which vitellogenesis commences, most of the growth in oocyte size occurs during late premoult. Thus, two distinct phases of vitellogenesis can be distinguished: The first phase is flexible in timing and can occur either in intermoult or during a protracted D_0; the second phase invariably occurs during late premoult. The occurrence of vitellogenesis during both intermoult and premoult also occurs in the amphipod *Orchestia* (Charniaux-Cotton, 1973; Meusy *et al.*, 1974), in which this arrangement has been ascribed to the short duration of intermoult relative to premoult (Adiyodi, 1978). However, vitellogenesis in isopods always continues into premoult even when intermoult is prolonged fourfold. This is suggestive less of insufficient time for the completion of vitellogenesis during intermoult than of different physiological requirements for the completion of the two phases.

The fact that a pause in late D_0 is seen only in the first maternal moult suggests that it is not a direct response to the occurrence of vitellogenesis but rather a response to the morphogenetic character of the moult. During a first maternal moult, the epidermis must become reorganized so that a brood pouch will be differentiated. Obviously, this reorganization must be completed before the commencement of cuticle deposition, *i.e.*, prior to the end of D_0. Thus, the protraction of D_0 may be required to effect this reorganization of epidermal cells for differentiation of a brood pouch. In contrast, during a second maternal moult, the first brood pouch is replaced by a second pouch and consequently no epidermal reorganization is required.

Synergism and antagonism. The relations between moulting and reproduction in crustaceans have been widely described as illustrating either "synergism" or "antagonism" between the two phenomena (references in Introduction). These two concepts are based almost entirely on the observation of either simultaneous or consecutive occurrence of moulting and reproduction in decapods. However, this terminology strongly implies that the temporal relations observed between moulting and reproduction are not merely circumstantial, but are a product of specific processes of mutual stimulation or inhibition. There is no compelling evidence of such processes, even in decapods. The present work suggests that this terminology is inappropriate, at least for isopods, and its implications potentially misleading.

The finding that moulting may occur either with or without reproduction according to daylength suggests that the two processes are independent but coordinated responses to environmental cues and are not simply "synergetic" or "antagonistic." Thus, the occurrence of vitellogenesis during premoult in isopods

is not interpreted as evidence of "synergism" between reproduction and moulting since there is no evidence that the occurrence of either process stimulates the other. The term "synchrony" as used to describe this phenomenon in *Orchestia* (Meusy *et al.*, 1977) has fewer functional implications and is considered preferable to "synergism."

Adiyodi and Adiyodi (1970) suggest that "antagonism" characterizes the reproductive period in decapods, on the supposition that the demands of the gonad for metabolic reserves interfere temporarily with the growth of the integument. While the pause observed in early premoult of isopods during which vitellogenesis commences could be construed as illustrating this notion, most of the oocyte growth was found to occur in late premoult when cuticle changes are proceeding concurrently. Hence, it is not the metabolic demands of the ovary which produce the pause in premoult. Similarly, the prolongation of intermoult during brood incubation could also be construed as "antagonism." However, there is no need to postulate an inhibition of moulting at this time; the initiation of premoult by sensory input from the brood pouch indicates coordination rather than "antagonism" between moult initiation and brood incubation.

Implications for hormonal control. Substantial evidence exists for many crustacean species of inhibitory neurohormonal control by the brain of both moulting and reproduction (reviewed by Gabe, 1966; Adiyodi and Adiyodi, 1970; Sochasky, 1973). There is continued debate over whether separate moult- and gonad-inhibiting hormones (MIH and GIH) occur or whether both processes are regulated by a single "growth inhibiting principle" (Panouse, 1947). As in decapods, there are conflicting reports concerning the effects of brain lesions on moulting and reproduction in isopods, (reference in Introduction). The present report is the first to examine the mechanisms coordinating moulting and reproduction in normal isopods. Certain requirements of any concept of hormonal control of these processes may now be inferred.

First, separate moult- and gonad-regulating hormones are necessary. All the above arguments that moulting and reproduction are separate but coordinated processes imply separate hormonal mechanisms; it is not possible to explain the interactions found between these processes in terms of a single "growth inhibitory principle." This conclusion is further supported by the finding that one of the groups of neurosecretory cells in the brain of *Oniscus* undergoes cytological changes correlated with the moult cycle while those of another group are correlated with vitellogenesis (unpublished observations).

Second, more than one hormone seems to be involved in vitellogenesis. Ecdysone appears to be necessary for vitellogenesis in *Porcellio* (Bessé and Maissiat, 1971). The present finding that vitellogenesis is always completed in late premoult, when whole body content of ecdysteroids peaks in other isopod species (Charmantier *et al.*, 1976; Hoarau and Hirn, 1978) suggests that ecdysone may be necessary for the completion of vitellogenesis. However, it is highly improbable that ecdysone initiates vitellogenesis, since it may commence either in intermoult or early premoult. Rather, its initiation may be controlled by some other principle (GIH or analogous factor), perhaps acting to stimulate production of vitellogenins. Regulation of the release of this principle by daylength would explain the observed effects of daylength on reproduction. The role of ecdysone might then be in the subsequent uptake of vitellogenins by the oocytes, as has been suggested in *Orchestia* (Meusy *et al.*, 1977; Blanchet *et al.*, 1979). The regulation of vitellogenesis

seems to be different in isopods and decapods, for the whole of vitellogenesis usually occurs during intermoult in the latter (Adiyodi and Adiyodi, 1970, for review).

Third, support is given to the proposal that isopod ovaries containing vitellogenic oocytes produce an "ovary hormone" which induces differentiation of a brood pouch (Legrand, 1955; Balesdent, 1965). During first maternal moults, the pause in late D_0 coincides with the beginning of vitellogenesis and ends with apolysis. Secretion of an "ovary hormone" would therefore commence at exactly the appropriate time to elicit the reorganization of the epidermis to form a brood pouch under the influence of ecdysone later in premoult. During second maternal moults, the ovaries contain vitellogenic oocytes at the commencement of premoult, enabling prompt secretion of hormone to ensure that the epidermis retained its ability to differentiate another brood pouch.

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Summary

In terrestrial isopods, different sensory cues initiate reproduction and moulting, indicating that the two processes are controlled by different physiological mechanisms. A specific sensory trigger which initiates premoult is identified; it occurs well before conventional signs of premoult become evident. Specific coordinating mechanisms adjust the chronology of moulting and vitellogenesis under conditions promoting both processes. The first phase of vitellogenesis can occur either in intermoult or early premoult according to conditions and is considered to be independent of ecdysone. The second phase invariably occurs in late premoult and may be ecdysone-dependent. The relations between moulting and reproduction are regarded as separately controlled processes which interact via specific cues which coordinate and adjust the timing of the two processes. Implications of this concept are discussed.

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